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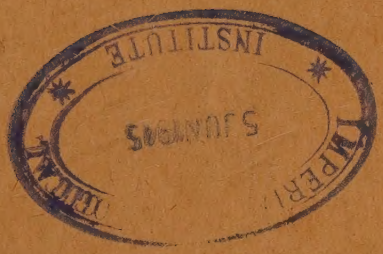

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF THE CHIEF,
Washington, D. C., January 29, 1902.

SIR: I have the honor to transmit herewith a paper entitled A Preliminary Study of the Germination of the Spores of *Agaricus campestris* and other Basidiomycetous Fungi, and respectfully recommend that it be published as Bulletin No. 16 of the Bureau series. The paper was prepared by Dr. Margaret C. Ferguson at Cornell University, and was submitted by the Pathologist and Physiologist.

Respectfully.

B. T. GALLOWAY,
Chief of Bureau.

Hon. JAMES WILSON,
Secretary of Agriculture.

PREFACE.

Recent investigations undertaken by Dr. B. M. Duggar, of this Office, show that there is a large and growing consumption of mushrooms for food in this country. As a rule the use of wild species of mushrooms is fairly safe where the collector knows which to take and which to avoid, but for the unskilled collector the cultivated product is much safer. This country imported last year about 3,000,000 pounds of canned mushrooms and practically all the spawn used, but there is no reason why we should not grow our own spawn and produce our own mushrooms. However, there are many difficult problems to be solved before the industry, which is comparatively new in this country, can be developed on the basis of accurate knowledge. The production of pure spawns of high vitality is one of the most important requirements, and should receive special attention. The accompanying paper is the result of work in this line undertaken and completed by Dr. Margaret C. Ferguson at Cornell University during the session of 1900–1901, under the direction of Dr. B. M. Duggar, then assistant professor of plant physiology in the university, and now a plant physiologist in this Office. Although technical, the paper bears directly on lines which are being developed in this Office, and will form the basis for future work having for its object the growing of pure virgin spawn. Work of this nature and its practical application are necessary for the better development of the mushroom industry in this country.

ALBERT F. WOODS,
Pathologist and Physiologist.

OFFICE OF THE PATHOLOGIST AND PHYSIOLOGIST,
Washington, D. C., January 29, 1902.

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A PRELIMINARY STUDY OF THE GERMINATION OF THE SPORES OF AGARICUS CAMPESTRIS AND OTHER BASIDIOMYCETOUS FUNGI.

INTRODUCTION.

During the past century many students have attempted, with varying degrees of success, to germinate the spores of the Basidiomycetous fungi. While some of these investigators have been able to germinate the spores of many of the Basidiomycetous fungi, their results with this group of plants have somehow failed to be of value in other departments of botanical research. The spores of the Basidiomycetes are seldom used to-day for cultural purposes. Neither have the successful experiments with edible forms become of widespread economic importance, for the French, who alone seem to have been successful in germinating the spores of the common edible mushroom, *Agaricus campestris*, keep their methods secret.

That the results of earlier investigators along this line have not received a wider application is probably due, in large measure, to the fact that they have treated this question almost exclusively from the standpoint of development and morphology. As will be seen from the history given at the close of this bulletin, the object of the student has been, as a rule, to germinate the spores that the life history of the plant might be studied. Thus the special conditions controlling germination in the Basidiomycetes have been almost totally neglected. In a recent paper by Duggar (1901), the question of spore germination has been considered in an entirely different way from that of the earlier investigators. His aim, as expressed in the introduction, was to determine in so far as possible the special factors which influence the germination of certain fungous spores.

It was for the purpose of learning somewhat more regarding the conditions under which germination occurs in the Basidiomycetes, and especially in *Agaricus campestris*, that this study was undertaken at the suggestion of Professor Duggar. The task has not proven an easy one, and after nine months of almost constant application much still remains to be done, although some results of considerable importance have been obtained. I regret, therefore, the necessity of publishing

at this point, but circumstances are such as to render it impossible for me to continue the work further.

As already indicated, this study has been carried on under the direction of Prof. B. M. Duggar at the botanical laboratory, Cornell University, and it is with pleasure that I acknowledge my indebtedness to him for his many helpful suggestions and his constant and kind assistance.

METHODS.

It will be readily understood that it is difficult—almost impossible—to obtain basidiospores in an absolutely pure condition. By observing certain precautions, however, it is possible to get these spores in such a state of purity that contaminated cultures occur but rarely in sterilized media, and never in sufficient number to be a serious hindrance to the study of germination.

Before collecting material, Petri dishes were prepared for the reception of the spores. After these dishes were thoroughly cleaned, white paper was fitted into the bottom and a piece of absorbent cotton placed over the top of each dish. The cover dishes were put on and the cotton was trimmed off with scissors until it was even with the outer edge of the covers. The dishes were then sterilized in a dry oven and set aside for later use. If it happened that several days elapsed before these dishes were needed, they were again sterilized immediately prior to their use.

Sporophores were collected just as the veil was about to break, when one was present, and again when the pileus was fully expanded, or nearly so. Spores from fruit bodies which showed traces of disintegration were not found to be satisfactory. The sporophores were wrapped in tissue paper and taken at once to the culture room. The margin and outer covering of the pileus were removed with sterilized forceps and scalpel. The pileus was cut into thirds or fourths, and the pieces, separated from the stipe with the forceps, were inverted in the previously prepared Petri dishes. During this process the forceps and scalpel were repeatedly resterilized in a Bunsen flame. The time required for obtaining good spore-prints varied from four to twelve hours, depending on the species and on the maturity of the fruit bodies when collected. After removing the pilei, the Petri dishes were packed away in a cool, dark place. In this manner spores were kept in good condition for many months. With gelatinous forms the spores were obtained by washing the mass with sterilized distilled water, and then rubbing bits of this mass on sterilized cover glasses by means of a platinum needle. These cover glasses were stored in Petri dishes in the same manner as were the spore-prints, and when tested for germination a drop of the culture medium was placed directly upon the smeared part.

The hanging drop cultures, so generally used in such work, were

found to be very satisfactory in this study. The methods employed in the use of the van Tieghem cell were such as have been described in detail by Clark (1899) and by Duggar (1901), and need not be repeated here. In a laboratory where several students are working upon physiological problems, the space in the thermostat which is available for each student often becomes much limited, and the question of economy in the use of that space comes to be a problem of considerable importance to the student. The writer has used a little device for supporting the slides which has proved to be very convenient. By means of it one can carry through a large number of cultures at once without making undue demands upon the thermostat. A description of this piece of apparatus is given in the appendix to this bulletin, with the thought that it may prove useful in general work.

The plant decoctions used as cultural media were made up according to the following formulas:

	Grams to one liter of water.
Green bean stems or pods	392
Sugar beets	370
Sporophores of *Calvatia cyathiforme*	300
Lepiota naucina (sporophores dried)	20
Pleurotus ostreatus (sporophores collected just after a rain)	400
Coprinus comatus, together with soil, grass, etc., in which it was growing	700
Fresh horse manure	85

A strong decoction of manure was also made from manure which had been fermented and was just ready to be used by the gardener in making up a mushroom bed.

The beef broth used was made after the formula ordinarily followed by bacteriologists.

The sugar solution was used at a concentration of $\frac{N}{10}$.

The distilled water employed in the experiments was always redistilled from a glass vessel and then sterilized.

Throughout the work the greatest care was taken to preserve the cultures free from contamination of any sort. Unless otherwise stated, the cultures were kept in a light-proof thermostat at a temperature of 28°± C. For the sake of uniformity in making up the cultures for a given test, large numbers of spores were first transferred from the spore-prints to a drop of distilled water on a sterilized cover glass. The cover was kept inverted over one of the van Tieghem cells, to which it was fastened with vaseline, during the intervals when spores were not being transferred from the drop to the cultures. In this way, too, the danger of contamination by repeatedly opening the Petri dishes containing the spores was reduced to a minimum. I have used the number 99 to indicate perfect or almost perfect germination. The large numbers of spores sown in each culture made it impossible to be sure that every spore had germinated, and hence the number 100 will not appear in any of the tables which are given in this report.

EXPERIMENTAL.

SPORE GERMINATION (PRELIMINARY STUDY).

In order to determine the desirability of certain forms for cultural purposes, preliminary tests were made on the germination of the spores of a number of species including representatives of several families of the true Basidiomycetes.

The results of this preliminary work are given in Table I. Of all the species observed, the spores of *Polyporus brumalis* undergo the most marked changes during germination. These spores swell to several times their original size and become almost spherical in outline before putting out germ tubes. Usually two and frequently three tubes are protruded from each spore. With the first experiments observations were made sixteen hours after the spores were sown, and then, as a rule, twenty-four and forty-eight hours later; but the necessity for running the cultures for a longer time gradually became apparent. Thus it came about that the time at which the last examinations were made varied much throughout this earlier work and is given in each instance in the table. In every case the highest percentage of germination shown by either duplicate at the time of the last examination of the cultures is the percentage recorded.

By a study of Table I certain comparisons may be made with regard to the amount of germination on various media and at different temperatures. Most of the conclusions, however, which may be drawn are to be considered as provisional only. More than one-third of the species studied germinated in distilled water, though none of them showed perfect germination in this medium. Ninety per cent, that recorded for *Phlebia radiata* and *Hypholoma appendiculatum*, was the highest percentage of germination observed in distilled water. *Coprinus micaceus*, reported by Duggar (1901) as not germinating in distilled water, gave in these tests 60 per cent of germination. With the exception of *Merulius tremellosus* and *Pholiota* sp., every form which germinated in any medium gave more or less germination in distilled water. It would appear that an external food supply is necessary for germination in these two species, while in the other forms showing germination it is not necessary, though it certainly enhances the power of germination in these spores, as shown by the higher percentages obtained in nutrient media. Distilled water gave almost invariably somewhat better germination than tap water.

TABLE I.—*Percentage of germination.*

Spores of—	Culture media.														
	Time in hours of last reading at—		Distilled water.		Tap water.	Sugar solution.		Bean decoction.		Beet decoction.	Lepiota decoction.		Calvatia decoction.	Coprinus decoction.	
			Temperature.		Temperature.	Temperature.		Temperature.		Temperature.	Temperature.		Temperature.	Temperature.	
	28°±C	16°±C	28°±C	16°±C	28°±C	28°±C	16°±C	28°±C	16°±C	28°±C	28°±C	16°±C	28°±C	28°±C	16°±C
Coprinus micaceus	48	160	60	25	70	85	33	99	99	80	98	98	75	95	99
Hypholoma appendiculatum	48	160	90	0	75	85	0	99	99	90	98	99	99	95	99
Collybia velutipes	64	160	80	2	30	99	0	99	90	99	99	65	99	99	90
Lepiota naucina	92	160	50	0	0	75	0	99	0	90	50	0	85	99	0
Pleurotus ostreatus	48	160	50	25	2	15	30	98	10	95	75	40	45	50	50
Tricholoma personatum	92	160	50	.1	0	0	.1	99	.1	25	99	8	90	99	8
Armillaria mellea	64	160	10	10	.01	75	10	0	.5	25	30	1	5	50	10
Polyporus brumalis	48	160	50	10	.05	99	20	99	99	99	99	99	85	99	75
Phlebia radiata	64	160	90	80	0	99	75	99	99	99	0	90	99	99	99
Merulius tremellosus	64	160	0	0	0	0	0	90	75	95	75	50	95	99	30
Dacryomyces corticioides	48	160	10	3	0	[a]	10	75	0	[a]	50	0	10	10	0
Coprinus comatus	92	160	10	.5	5	0	6	0	5	0	5	5	0	0	4
Hypholoma sublateritium	48	160	0	10	0	0	2	40	85	0	10	50	10	40	90
Clitocybe candida	48	160	.01	2	.01	0	0	0	0	0	25	20	10	0	5
Pholiota	92	160	0	0	0	0	0	25	0	1	5	0	50	40	0
Coprinus atramentarius	64	160	0	0	0	0	.1	0	0	0	0	0	0	0	0
Agaricus campestris	92	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Agaricus placomyces	92	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepiota cristata	64	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricholoma terreum	92	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurotus sapidus	92	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Pholiota squarrosoides	64	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Calvatia cyathiforme	92	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycoperdon pyriforme	64	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycoperdon Wrightii	48	160	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycoperdon gemmatum	92	160	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] One spore.

The inhibiting effects on germination of a temperature of 16° ± C. was much more marked in distilled water and sugar solution than in the other media. Heald (1898) has shown that in the case of fern spores an increase in temperature can be substituted for the stimulus of light, and it is evident from my experiments that in some of the Basidiomycetes a high temperature is the only special stimulus required to produce germination. It is rather significant that while *Hypholoma appendiculatum* gave 90 and 75 per cent germination at 28° ± C. in distilled water and sugar solution, respectively, there was no germination in these media at a temperature of 16° ± C. This test was repeated three times with duplicates, and in only one case was there any germination in these media at the lower temperature. In this instance 15 per cent of germination occurred after 164 hours in distilled water and 20 per cent in sugar solution. In the same tests all the other media gave almost perfect germination at both the higher and the lower temperature. The effect of a high temperature in the absence of all food, or when a carbohydrate alone is present, is certainly very marked in this case.

In all the forms which showed germination, with the exception of *Hypholoma sublateritium*, 16° C. is below the optimum temperature for germination. Germination was not only much retarded by this lower temperature, sometimes having been delayed for several days, but the final percentages were as a rule slightly lower. In many cases spores which had not germinated after having been kept at the lower temperature for one week germinated in twenty-four hours when transferred to the thermostat at 28° C. In the light of later results, there can be little doubt that some of the species, which gave no germination in these preliminary tests, would have germinated had the cultures been kept for a longer time.

For further study of the physiology of germination, *Agaricus campestris*, *Agaricus placomyces*, *Coprinus comatus*, *Lepiota naucina*, *Hypholoma appendiculatum*, *Polyporus brumalis*, *Merulius tremellosus*, *Phlebia radiata*, *Calvatia cyathiforme*, and *Lycoperdon pyriforme* were selected. This list includes species which had given almost perfect germination, others which had yielded various small percentages of germination, and still others which had been entirely resistant to the cultural conditions. With such a set of spores valuable results ought to be obtained regarding the stimulating or inhibiting effects of certain substances or conditions.

EXTREMES OF TEMPERATURE.

A large number of experiments were made with the fungi listed above to test the effects of extremes of temperature on their germination. This was suggested by the studies of Haberlandt (1878), Müller-Thurgau (1885), and Eriksson (1895). These investigators found that subjection to cold for a longer or shorter period not only im-

proved the germinating power of certain seeds and spores, but affected favorably other life processes as well.

The spores to be tested were put into a few drops of distilled water in tiny vials which had been previously plugged with cotton and sterilized. Several sets of vials were made up and subjected to the following treatment before the spores were transferred to the cultures:

No. 1. Cold.[a]
No. 2. Cold + heat.[b]
No. 3. Cold + heat + cold.
No. 4. Heat.
No. 5. Heat + cold.
No. 6. Heat + cold + heat.

While the exposure to a high temperature was always for ten minutes, the spores were tested after having been in the cold for different lengths of time varying from one day to three weeks.

TABLE II.—*Percentage of germination after 168 hours.*

Temperature 28° ± C.

Spores of—	Spores subjected to—	Culture media. Distilled water.	Sugar solution.	Bean decoction.	Lepiota decoction.	Coprinus decoction.
Hypholoma appendiculatum	A. Cold 1 day.	95	95	99	99	99
Polyporus brumalis		1	40	99	99	99
Merulius tremellosus		0	0			
Phlebia radiata		0	95	95	85	98
Lepiota naucina		0	25	50	10	15
Coprinus comatus		0	0	0	0	0
Agaricus campestris		0	0	0	0	0
Agaricus placomyces		0	0	0	0	0
Calvatia cyathiforme		0	0	0	0	0
Lycoperdon pyriforme		0	0	0	0	0
Hypholoma appendiculatum	B. Cold 6 days.	99	99	99	99	99
Polyporus brumalis		2	25	99	99	99
Merulius tremellosus		0	10	90	80	75
Phlebia radiata		50	50	99	75	99
Lepiota naucina		10	25	90	50	50
Coprinus comatus		0	0	0	0	0
Agaricus campestris		0	0	0	0	0
Agaricus placomyces		0	0	0	0	0
Calvatia cyathiforme		0	0	0	0	0
Lycoperdon pyriforme		0	0	0	0	0
Hypholoma appendiculatum	C. Heat.	95	95	99	99	99
Polyporus brumalis		25	30	99	99	99
Merulius tremellosus		10	5	90	98	85
Phlebia radiata		15	50	98	99	99
Lepiota naucina		15	50	99	99	95
Coprinus comatus		0	0	0	0	0
Agaricus campestris		0	0	0	0	0
Agaricus placomyces		0	0	0	0	0
Calvatia cyathiforme		0	0	0	0	0
Lycoperdon pyriforme		0	0	0	0	0
Hypholoma appendiculatum	D. Heat + cold 2 days.	90	98	99	99	99
Polyporus brumalis		5	99	99	99	99
Merulius tremellosus		25	95	99	99	99
Phlebia radiata		40	95	99	99	99
Lepiota naucina		40	90	98	98	98
Coprinus comatus		0	0	0	(c)	0
Agaricus campestris		0	0	0	0	5
Agaricus placomyces		0	0	0	0	0
Calvatia cyathiforme		0	0	0	0	0
Lycoperdon pyriforme		0	0	0	0	0

[a] By "cold" it will be understood that the vials containing the spores were placed outside a north window in January. During the times of exposure the temperature ranged from 10° C. in the daytime to —5° C. at night. Usually, however, the temperature did not rise to more than 3° or 4° C. during the day.

[b] "Heat" indicates that the spores were heated to 42° C. in distilled water for ten minutes.

[c] 1 spore.

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The records of the results obtained in the first four tests with extremes of temperature are to be found in the second table. This gives substantially all that there is to be learned from the whole series of temperature experiments. The spores were taken from the same spore print of each species in all four tests. By a comparison of these records certain conclusions seem evident. A longer exposure to cold is in most instances a better stimulus to germination than a shorter exposure; the deleterious effect of cold on germination is much more pronounced when no external food supply is present. To illustrate, it will be sufficient to compare the records made by two species at 28° C. as shown in Table I, and in sections A and B of Table II.

		Distilled water.	Bean decoction.
		Per cent.	*Per cent.*
Lepiota naucina	I	50	99
	II, A	0	50
	II, B	10	90
Phlebia radiata	I	90	99
	II, A	0	95
	II, B	50	99

A short exposure to a high temperature seems to be a stimulus to germination, but heat followed by cold acts as a slightly stronger stimulant. It will be noted that the spores of *Merulius tremellosus* did not germinate in distilled water until after having been subjected to a temperature of 42° for ten minutes, and that the spores of *Agaricus campestris* first germinated under the combined action of heat and cold. It does not appear, however, after a study of several hundred cultures in which the spores had been subjected to extremes of temperature, that such treatment is of any marked advantage in the germination of the spores of *Agaricus campestris*.

It was found that heating in water at 52° C. for ten minutes was sufficient to kill the spores, at least of those forms which had germinated under other conditions. Also, repeated freezings and thawings in water during a period of three months destroyed the vitality of the spores. Later on in the work spores of *Agaricus campestris* were several times put into distilled water and submitted to the action of a freezing mixture, temperature—18° C., for from ten to twenty minutes. Only in very rare instances did such spores germinate after being transferred to the culture media. Frequently the spores lost their characteristic appearance and showed undoubted evidence of having been injured by the extreme cold.

ACTION OF AN ARTIFICIAL DIGESTIVE FLUID.

It is well known that *Agaricus campestris* occurs naturally in stable yards and in pastures, and it is generally supposed that the spores germinate in these situations after having passed through the digestive

tract of herbivorous animals. Janczewski (1871) was able to prepare the spores of *Ascobolus furfuraceus* for germination only by feeding them to rabbits, and it was suggested by Professor Duggar that the action of an artificial digestive fluid might have the same effect. Spores of *Agaricus campestris*, *Coprinus comatus*, and *Calvatia cyathiforme* were used in these tests.

Five-tenths and one-tenth per cent solutions of pepsin in distilled water were combined with hydrochloric acid at a concentration of $\frac{N}{100}$, $\frac{N}{1000}$, and $\frac{N}{10000}$, also in distilled water. A few drops of each concentration were put into vials, spores were added, and the vials after being sealed were placed in the thermostat. After 24 hours, at the end of 3 days, and again after 7 days, spores were transferred to culture drops of distilled water, and of decoctions of manure, Coprinus, and bean pods. The experiments were repeated three times with four duplicates each time, and the cultures were kept for 8 days. *Calvatia cyathiforme* and *Coprinus comatus* showed no germination. Several of the cultures of *Agaricus campestris* gave various small percentages of germination; but no germination occurred in distilled water nor in the larger number of the other cultures. One culture in Coprinus decoction registered 25 per cent, the highest percentage of germination which had been observed in *Agaricus campestris* up to that time. The spores of this culture had been treated with $\frac{N}{10000}$ HCl and one-tenth per cent solution of pepsin for 7 days before they were transferred to the hanging drop. But in the three duplicate cultures, and in fact in all the other cultures of this experiment, not a single spore was observed to germinate. With the other two experiments frequently from 10 to 20 per cent of the spores of one of the four duplicate cultures germinated; in other instances two, three, and occasionally all four of the duplicate cultures showed some germination. Such irregularities are not peculiar to the tests with an artificial digestive fluid, but are characteristic of nearly all the experiments made with the spores of *Agaricus campestris*. I am wholly unable to account for these erratic results. It would seem that the state of maturity or other conditions of the spores must be the cause; but that other factors are also influential in producing them there can be little doubt in the light of later experiments. On the whole, there was somewhat more germination in the cultures containing $\frac{N}{1000}$ and $\frac{N}{10000}$ HCl than with those with $\frac{N}{100}$ HCl; but it seemed a matter of indifference whether one-half or one-tenth per cent of the pepsin was present.

Temperature tests as previously described were combined with the action of this artificial digestive fluid, but the amount of germination was not thereby appreciably increased. Germination occurred in a greater number of cultures with this treatment, but the percentages rarely exceeded 12. Other experiments were made in which the hydrochloric acid and pepsin were put directly into the various media used in the cultures. The results were not substantially different from those obtained by subjecting the spores to the combined action of hydrochloric acid and pepsin for different lengths of time, and then transferring them to drops of pure decoction.

EFFECT OF ACIDS ON GERMINATION.

Hoffman (1860) found that weak acids did not exercise a stimulating effect upon the germination of Basidiomycetous spores. And inasmuch as the experiments with hydrochloric acid and pepsin had given only small percentages of germination, it was thought that the acid might have an inhibiting action on these spores. We therefore tried the effect of this acid on spores that were known to germinate abundantly in media not acid. The results are recorded in Table III.

The conditions governing germination in *Agaricus campestris* seemed so complex and subtle that the other species were dropped at this point, and the investigations thenceforth were directed almost entirely to the problems of germination in this economic species.

Experiments with malic, lactic, and hippuric acids were repeated several times. The results given in Table IV are fairly typical. There was never any germination in malic acid, but both lactic and hippuric acids gave small percentages of germination—now in one medium, now in another. Spores which had been heated in $\frac{N}{1000}$ HCl and one-half per cent pepsin in distilled water, and then exposed to outside temperatures from January 5 to January 29, were transferred to cultures of manure and Coprinus decoctions containing hippuric acid. Positive results were obtained in 10 out of 14 cultures in 168 hours. This was the most uniform germination that had yet been obtained with *Agaricus campestris*, but in no case did the percentage of germination exceed 15.

TABLE III.—*Percentage of germination after 1 day.*

[Temperature 28 ± C.]

Spores of—	HCl in Lepiota decoction.				
	$\frac{N}{300}$ HCl.	$\frac{N}{500}$ HCl.	$\frac{N}{1000}$ HCl.	$\frac{N}{5000}$ HCl.	$\frac{N}{10000}$ HCl.
Coprinus micaceus	25	25	20	50	70
Hypholoma appendiculatum	95	98	97	97	98

TABLE IV.—*Percentage of germination after 10 days.*

AGARICUS CAMPESTRIS.

Temperature 28 ± C.

Medium.	4 drops of N malic acid[a] in—					
	2 cc. of decoction.	4 cc. of decoction.	6 cc. of decoction.	8 cc. of decoction.	10 cc. of decoction.	12 cc. of decoction.
Coprinus decoction	0	0	0	0	0	0
Bean decoction	0	0	0	0	0	0

Medium.	4 drops of N lactic acid[a] in—					
	2 cc. of decoction.	4 cc. of decoction.	6 cc. of decoction.	8 cc. of decoction.	10 cc. of decoction.	12 cc. of decoction.
Coprinus decoction	0	20	[b]1.5	[c]2	[d]5	0
Bean decoction	0	0	0	0	0	0

Medium.	10 drops of a saturated solution of hippuric acid[a] in—					
	2 cc. of decoction.	4 cc. of decoction.	6 cc. of decoction.	8 cc. of decoction.	10 cc. of decoction.	12 cc. of decoction.
Coprinus decoction	0	0	0	0	0	0
Bean decoction	0	0	0	0	10	8

[a] The decoctions were neutralized before the acids were added.
[b] 75 spores. [c] 95 spores. [d] 250 + spores had germinated.

ACIDS FOLLOWED BY ALKALIES.

After six days the spores which had not germinated in the acid cultures were, in many cases, transferred to cultures of similar decoctions containing varying percentages of ammonium hydrate, sodium carbonate, and potassium hydrate, respectively. These gave only negative results, as did also the experiments in which fresh spores were put into several different concentrations of the above named acids in distilled water, and subjected to extremes of temperature before being sown in alkaline media.

EFFECT OF LIGHT ON GERMINATION.

Spores were sown in cultures of various media, and in the presence of all the different stimuli which had yielded germination in the dark in any culture throughout this entire study. One set of these was placed in direct sunlight and another in diffuse light. After 4 weeks no germination had occurred in any of the cultures. This would seem to indicate that light has an inhibitory effect on the germination of these spores; but the point needs further investigation.

AGE OF THE SPORES RELATIVE TO THEIR POWER OF GERMINATION.

No exhaustive study has been made regarding the effect of age on the germination of the spores of *Agaricus campestris*. But incidentally it has been demonstrated that the spores will germinate if placed in cultures on the same day in which the spore-prints have been obtained. Spores 6 months old have also been brought to germination, but spores older than this have not been tested. We are, there-

fore, unable to say during how long a period these spores may retain their vitality. The results obtained in this study show very conclusively, however, that, other things being equal, the comparatively fresh spores are much more satisfactory for cultural purposes than are the older ones.

A NEW FACTOR IN GERMINATION.

During the greater part of this work it was my practice to discard cultures after from 200 to 240 hours. As a rule, observations were made at the end of 72, 96, 144, 168, and 240 hours, respectively. If germination occurred at all it usually appeared in about 7 days, but in a few cases 4 days sufficed. This was followed by a rapid growth; but no signs of further germination being apparent at the close of 10 days, there seemed no reason why these cultures should not be replaced by other experiments. The spores from those cultures in which partial germination had occurred were transferred at this time to test tubes of sterilized manure, bean stems, and other solid substrata.

At one time in the early spring, after 250 cultures had been running for the usual number of days, it was felt that it might be well to keep them for a longer time. They were again examined after 336 hours when, to my great surprise, I found almost perfect germination in some of the cultures. This set of cultures was kept for 4 weeks and examined 11 times, but there was no apparent change after 384 hours. A few of the results obtained are given in Table V. In the light of these observations, all previous work seemed to require repetition. By way of comparison, some of the results from an experiment of about 500 cultures which followed the test referred to above are given in Table VI. The spores in the first 2 cultures in each decoction, and in each concentration of the particular stimulus used (Table V), were from a spore-print 55 days old. The last 2 cultures in every instance in Table V contained spores which had been obtained but 7 days prior to being sown. Spores from this same spore-print were used in the first 2 cultures of every medium in Table VI, but were sown more than a month later. The other cultures in this last experiment contained spores from a spore-print but 12 days old.

We would not have it understood from these tables that in just 9 days after sowing, some spores germinated, and that on the twenty-first day 95 per cent, more or less, of germination occurred. Some spores had emitted germ tubes after 4 days, others after 7, and still others not until the end of 14 days. Hence "9 days" is used as an average time for the first appearance of germination. The "21 days" gives the results as recorded at the last examination of the cultures. The cultures were examined 11 times, but it would not be well to complicate these tables by reporting all the readings. In every case where a high percentage of germination has finally occurred it has been preceded a few days by the germination of 1 or more spores. The

germ tubes of the first spores germinating are very broad (Pl. I, fig. 1), and, if conditions are favorable for their growth, they develop rapidly, ramifying in every direction (Pl. I, fig. 6). From 5 to 10 days later almost every spore in such cultures germinates, forming 1 or 2 short, almost spherical tubes (Pl. I, fig. 2). It has never happened in these cultures that 40 per cent of the spores germinate, then 75 per cent, and later 99 per cent; but after the hyphae from a few spores of *Agaricus campestris* have developed to a greater or less degree, all other spores in the culture may send out germ tubes, if not simultaneously, certainly at about the same time. In some cases it has happened that a single spore has germinated at one side of the culture and grown toward the mass of spores collected at the center of the drop. Just as it has reached this mass nearly every spore, as by magic, has emitted a germ tube.

The germination of a few spores does not insure fuller germination under all circumstances. Some spores have germinated in several cultures containing asparagin, but these have shown little growth, and never any further germination; the same phenomenon has been observed in many cultures and various media. But I have never seen anything like perfect germination which has not been preceded by a considerable growth, in the culture, of the mycelium of *Agaricus campestris*.

Attention is called to the fact that in the presence of ammonium nitrate 60 per cent of the spores finally germinated in one culture in distilled water (Table VI). This is the highest percentage of germination observed for *Agaricus campestris* in distilled water in the presence of any stimulus tested. In this instance there was considerable growth from the 2 spores which germinated first, hence the fuller germination. Whether the ammonium nitrate was sufficient of itself to nourish for a time the growing hyphae, or whether in its presence the organic substance of some of the other spores became available as food, remains an open question. That the spores contain within themselves sufficient food for the first stages of growth following germination seems doubtful in view of the fact that when a large number of spores germinate in a given culture there is no further growth unless the spores are transferred to drops of fresh decoction.

The erratic results characteristic of all the cultures of *Agaricus campestris* are well illustrated by the records given in Tables V and VI. In the check, 1 culture of the 4 duplicates in each decoction yielded over 90 per cent germination, and the other 3 gave absolutely none. In the first experiment 12 cultures with ammonium hydrate registered a percentage of from 80 to 99, and not a single spore in lactic acid germinated. But in the second test exactly the opposite was true. No germination occurred in the presence of ammonium, while there was more than 90 per cent germination in several of the cultures with lactic acid.

TABLE V.—*Percentage of germination.*

Temperature 28° ± C.

AGARICUS CAMPESTRIS.

Culture media.	Check.		NH_4OH						KOH					
			$\frac{N}{100}$		$\frac{N}{1000}$		$\frac{N}{10000}$		$\frac{N}{100}$		$\frac{N}{1000}$		$\frac{N}{10000}$	
	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.
Bean decoction	0	0	0	0	0	0	3 spores	90	0	0	0	[a]35	0	0
	0	0	0	0	3 spores	98	1 spore	0	0	0	0	0	0	0
	1 spore	98	0	10	1 spore	80	0	.....	0	0	0	0	0	0
Beet decoction	0	0	1 spore	98	0	0	2 spores	2 spores	0	1 spore	0	0	0	0
	0	0	0	0	6 spores	50	0	0	0	0	0	0	0	0
	0	0	0	0	1 spore	.....	2 spores	80	0	0	0	0	0	0
Manure decoction	0	0	1 spore	2 spores	0	0	0	0	0	0	2 spores	98	0	0
	1 spore	90	0	0	0	0	11 spores	99	0	0	0	0	0	0
	0	0	2 spores	99	7 spores	85	1 spore	99	0	2 spores	0	0	0	0
	0	0	1 spore	2 spores	3 spores	.....	0	1 spore	0	0	0	0	2 spores	55
	0	0	7 spores	99	0	0	2 spores	95	1 spore	1 spore	0	0	0	0
	1 spore	99	2 spores	2 spores	4 spores	90	2 spores	80	0	0	0	0	0	0
	—17° for 10 min.		Lactic acid.						Hippuric acid.					
Bean decoction	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Beet decoction	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Manure decoction	0	0	0	0	0	0	0	0	10 spores	40	0	0	0	0
	0	0	0	0	0	0	0	0	3 spores	4	0	0	0	0
	0	0	0	0	0	0	0	0	.....	0	0	0	0	0
	0	0	0	0	0	0	0	0	120 spores + 3 per cent.	0	0	0	0	0

[a] Four days after a few germinated spores from another culture had been transferred to this culture.

TABLE VI.—*Percentage of germination.*

Temperature 28° ± C.

AGARICUS CAMPESTRIS.

Strength of solution.	NH_4OH in—								NH_4NO_3 in—							
	Distilled water.		Bean decoction.		Manure decoction.		Lepiota decoction.		Distilled water.		Bean decoction.		Manure decoction.		Lepiota decoction.	
	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.	9 days.	21 days.
N/100	0	0	0	0	.....	.....	.....	.....	.....	.....	1 spore	98	.....	.....	.....	.....
	0	0	0	0	.....	.....	.....	.....	.....	.....	0	0	.....	.....	.....	.....
	0	0	0	0	.....	.....	.....	.....	.....	.....	4 spores	50	.....	.....	.....	.....
	0	0	0	0	.....	.....	.....	.....	.....	.....	0	0	.....	.....	.....	.....
N/1000	0	0	0	0	0	0	.....	.....	0	0	0	0	0	0	.....	.....
	0	0	0	0	0	0	.....	.....	0	0	0	0	0	0	.....	.....
	0	0	0	0	0	0	.....	.....	0	0	0	0	1 spore	1 spore	.....	.....
N/10000	0	0	0	0	0	0	0	0	2 spores	60	0	0	0	0	7 spores	7 spores
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3 spores	95
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4 spores	25
	0	0	0	0	0	0	0	0	0	0	0	0	5 spores	5 spores	0	0

	Lactic acid.								Asparagin.							
N/100	.....	.....	15 spores	100 spores +1.5 per cent.	.....	.....	.....	.....	.....	.....	0	0	.....	.....	.....	.....
	.....	.....	0	0	.....	.....	.....	.....	.....	.....	0	0	.....	.....	.....	.....
	.....	.....	0	0	.....	.....	.....	.....	.....	.....	0	0	.....	.....	.....	.....
	.....	.....	0	0	.....	.....	.....	.....	.....	.....	0	7 spores	.....	.....	.....	.....
N/1000	50 spores + 100 spores +	50 spores + 800 spores +=2 per cent.	100 spores + 33 spores	99 98	0 28 spores	0 98	.....	.....	3 spores 0	5 spores 0	1 spore 0	2 spores 0	0 0	0 0	.....	.....
	0	0	0	3 spores	4 spores	4 spores	.....	.....	0	0	0	0	0	0	.....	.....
	0	0	0	3 spores	2 spores	98	.....	.....	2 spores	5 spores	2 spores	2 spores	0	0	.....	.....
N/10000	5 spores 100 spores +	5 spores 500 spores +=1 per cent.	11 spores 18 spores	95 99	0 3 spores	0 3 spores	45 spores 23 spores	95 40	0 0	0 1 spore	2 spores 0	3 spores 0	0 0	0 0	3 spores 0	3 spores 0
	0	0	2 spores	10 spores	1 spore	1 spore	3 spores	99	0	0	0	0	0	0	5 spores	5 spores
	0	0	0	1 spore	0	0	9 spores	75	0	0	3 spores	3 spores	0	0	3 spores	3 spores

I can suggest no entirely satisfactory explanation of these variations. The utmost care was taken, not only to avoid organic contamination, but, also, in the use of pure chemicals and clean dishes. A sufficient amount of each medium was made up at the beginning of the work to last throughout the year, and a given medium was not stored in one large flask, but in several small ones to avoid change by repeated sterilizations. It seems impossible, therefore, to account for the erratic results by impurities or variations in the culture media. And to say that the cause lies wholly in the spores is equally unsatisfactory. When making up the cultures for a given test, the spores, as described earlier, were thoroughly mixed in a drop of distilled water before being transferred to the hanging drops. Under such conditions it seems extremely improbable that all the spores transferred to one medium should be incapable of further development, while large numbers of those transferred to another medium should be capable of germination. But after all it can not be doubted that an important factor in this problem is to be found in the condition of the spores themselves.

In this connection it has been observed that the spores from one spore-print have given more uniform germination than those from any of the many other spore-prints tested. In this case the fungus was raised in the conservatory of the Department by Mr. Shore, and the large, beautiful sporophore was taken immediately after the pileus had expanded. Large numbers of spores have been sown in each culture from the first; for, only small percentages of germination having been obtained, it was thought that the condition of maturity of the spores might largely control results. And in case no spores capable of germination were sown, one might conclude that the special stimulus tested had inhibited germination. The massing of the spores had no stimulating effect, as spores scattered through the drop germinated quite as frequently as those massed at the center. It is probable that the irregularities thus far recorded were due, in some measure, to the fact that up to this point in our study we had not found the best stimulus for the germination of these spores.

EFFECT OF MYCELIUM ON GERMINATION.

Mention has been made of transferring the spores from cultures which had germinated to a solid substratum in test tubes. In some cases, especially on bean stems, an abundant growth of mycelium resulted, and was available for the study of the effect of the mycelium on germination. The results of these experiments were very conclusive, as is shown by a glance at Table VII. This table records the readings for a single decoction, but many other decoctions were used with similar results. Where there was no mycelium present a few spores germinated in some cultures after 240 hours, perfect germination occurring only after 384 hours, but cultures into which bits of the

mycelium had been introduced gave almost perfect germination in 144 hours. In some decoctions containing a bit of the mycelium germination did not occur until after 168 hours. In all cases the presence of the growing mycelium caused a high percentage of germination at least one week sooner than it might otherwise have occurred, that is, by the germination of a few spores the mycelium of which would act as a stimulus.

TABLE VII.—*Percentage of germination.*

Temperature 28°± C.

AGARICUS CAMPESTRIS.

Culture media.	Hours.						
	96	144	168	240	312	384	504
Manure decoction	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	3 spores.	3 spores.	99	99
	0	0	0	0	0	0	0
Manure decoction, with a bit of the mycelium of Agaricus campestris	0	98	98	98	98	98	98
	0	95	95	95	95	95	95

In no cultures in which the growing mycelium of *Agaricus campestris* has not been present has a high percentage of germination ever occurred in any of the tests made in connection with this study. It is very evident that the growing mycelium in some manner prepares the way for germination. As to just how this is done it is, perhaps, vain to conjecture at present, but it is not improbable that some secretion is formed which stimulates or makes possible the emission of the germ tubes. Hartig (1894) has found that the presence of ammonium or some other alkali is necessary for the dissolving of the "spore pellicle" before germination can take place in *Merulius lachrymans*.

De Bary (1884) stated that the necessity of oxygen for the germination of fungous spores has never been sufficiently demonstrated; and it was thought that the phenomenon under discussion might be due to the fact that the great majority of these spores were able to germinate only after the oxygen of the hanging drop had been exhausted. We have not been able to test this point experimentally; but, if the presence or absence of oxygen were the controlling agent here, it would seem that the growth of the mycelium of any other fungus would affect germination as favorably as that of *Agaricus campestris* itself. This, however, is not the case, as mentioned later.

It has been determined, then, beyond a question, *that if a few spores are able to germinate under the cultural conditions, or if a bit of the mycelium of Agaricus campestris be introduced into the culture, the growth resulting will in either case cause or make possible the germination of nearly all the spores of the culture, provided, of course, that the other conditions are not such as to inhibit germination.* Mycelium that is not growing, or masses of spores, have not been

observed to influence germination. Tests with the growing mycelium of Mucor, Penicillium, *Coprinus micaceus*, and *Hypholoma appendiculatum* have yielded only negative results. In one culture, however, with the mycelium of *Hypholoma appendiculatum* about 50 per cent germination occurred, but there is a possibility that in this instance one or more spores of *Agaricus campestris* germinated first and were obscured from view by the mycelium which had been introduced into the culture. The presence of the mycelium of these forms does not inhibit germination, for if a few spores emit germ tubes in the cultures with them, the fuller germination follows in due time, as when no foreign fungus is present. Spores of *Coprinus micaceus* and *Hypholoma appendiculatum* were sown in cultures with the spores of *Agaricus campestris*. They germinated after a few hours and grew abundantly, but at the end of three weeks the spores of *Agaricus campestris* had given no evidence of germination.

The effect of the presence of the growing mycelium of *Agaricus campestris* on the spores of *Agaricus placomyces* and *Calvatia cyathiforme* was also tested. After several weeks there was not the least sign of germination in any of the cultures.

The germ tubes which are emitted under the influence of the growing mycelium of *Agaricus campestris* are short, thick, and, as a rule, almost spherical in outline (Pl. I, fig. 2). Little or no growth has ever been observed to follow this later germination, even when the cultures have been kept for several weeks. But if these spores are transferred to fresh media, growth begins at once. (Pl. I, figs. 3 and 4.)

A list of substances tested with regard to their effect on the germination of the spores of Agaricus campestris.

THOSE YIELDING POSITIVE RESULTS, OR, AT LEAST, OCCASIONALLY YIELDING POSITIVE RESULTS.

Distilled water.

Bean decoction.

Beet decoction.

Lepiota decoction.

Calvatia decoction.

Decoction of *Coprinus comatus* together with the soil in which the fungi were growing.

Decoction of fermented stable manure.

Decoction of fresh horse manure.

Lactic acid in distilled water, and in decoctions of bean, beet, Coprinus, Lepiota, and manure.

Hippuric acid in decoctions of bean, manure, and Coprinus.

Hydrochloric acid + pepsin in distilled water, and in bean, Coprinus, and manure decoctions.

Hydrochloric acid + pepsin + hippuric acid in distilled water, and in decoctions of bean, Coprinus, and manure.

Ammonium in bean, beet, and manure decoctions.

Potassium hydrate in bean, beet, and manure decoctions.

Ammonium nitrate in distilled water, and in decoctions of bean, Lepiota, and manure.

Potassium nitrate in bean and manure decoctions.

Asparagin in distilled water, and in bean, Lepiota, and manure decoctions.

Mycelium of *Agaricus campestris* in distilled water, and in decoctions of bean, beet, Lepiota, Coprinus, beef, and manure.

Bean agar.

Manure agar.

SUBSTANCES YIELDING NEGATIVE RESULTS.

Sugar solution.

Beef decoction.

Pleurotus decoction.

Glycerin.

Malic acid in Coprinus and bean decoctions.

Hippuric acid in distilled water and in beet decoction.

Hydrochloric acid + pepsin in Lepiota decoction.

Hydrochloric acid + pepsin + hippuric acid in Lepiota decoction.

Malic acid followed by potassium hydrate in bean, Coprinus, and manure decoctions.

Malic acid followed by ammonium in bean, Coprinus, and manure decoctions.

Malic acid followed by sodium carbonate in bean, Coprinus, and manure decoctions.

Lactic acid, followed by potassium hydrate in bean, Coprinus, and manure decoctions.

Lactic acid followed by ammonium in bean, Coprinus, and manure decoctions.

Lactic acid followed by sodium carbonate in bean, Coprinus, and manure decoctions.

Malic acid followed by hydrochloric acid + pepsin in bean, Coprinus, and manure decoctions.

Lactic acid followed by hydrochloric acid + pepsin in bean, Coprinus, and manure decoctions.

Alcohol in bean, beet, and manure decoctions.

Ether in bean, beet, and manure decoctions.

Ammonia in distilled water and in Lepiota decoction.

Potassium hydrate in distilled water and in Lepiota decoction.

Potassium nitrate in distilled water and in Lepiota decoction.

Potassium sulphate in distilled water and in Lepiota and bean decoctions.

Potassium carbonate in distilled water and in decoctions of bean, Lepiota, and manure.

Sodium sulphate in distilled water and in decoctions of bean, Lepiota, and manure.

Spores sown between cover glass and filter paper moistened with bean, beet, Coprinus, and manure decoctions.

Spores subjected to pressure by being placed in drops of manure, and of Lepiota decoction between larger cover glasses.

Mycelium of Mucor in distilled water, and in bean, beet, Coprinus, Lepiota, Lycoperdon, and manure decoctions.

Mycelium of Penicillium, as for Mucor.

Mycelium of *Coprinus micaceus* in Lepiota and manure decoctions.

Mycelium of *Hypholoma appendiculatum* in Lepiota and manure (?) decoctions.

Germinating spores of *Coprinus micaceus* in Lepiota and manure decoctions.

Germinating spores of *Hypholoma appendiculatum* in Lepiota and manure decoctions.

A high percentage of germination has, perhaps, been more frequently obtained with cultures in pure Lepiota decoction than in any other decoction used. Of the chemical stimuli tested, ammonium compounds and lactic acid seem to be the most effective.

It must not be inferred when no germination has occurred in a given medium or in the presence of certain stimuli that these substances necessarily have an inhibitory action. They may have, but because of the very erratic nature of the results thus far obtained a much more extended study of this subject must be made before the fact of such inhibition can be definitely established, for, although between 4,000 and 5,000 cultures have already been made, we are not yet in a position to draw final conclusions. This would not be considered a large number of cultures if one were working with the molds where tests can be carried through in three days, or in less time. But when studying a form which must be kept in culture for three weeks, and repeatedly examined, it becomes a much more arduous task. When cultures require to be kept for a long time, frequent readings may not seem necessary; but it is much more satisfactory not to allow too long a period to elapse between the examinations of the cultures. In fact, because of contaminations and other irregularities that may appear in such cultures, frequent observations are absolutely necessary if results are to be reliable.

So far as the writer is aware there is recorded here the highest percentage of germination yet observed in *Agaricus campestris*. Judging from Repin's (1898) statement, "The spores which germinate, and these are always the fewest in number, * * * " the French have not obtained anything like perfect germination, and no record has been found of the germination of these spores by other students.

The results which we have obtained would seem to indicate that the problems involved here must be very complex and subtle. And while this study throws much light on the questions at issue, it can be considered as scarcely more than preliminary to the work which must follow before the secrets which *Agaricus campestris* has guarded so closely through the ages are revealed. Professor Duggar has already made arrangements for carrying on this work, not only to determine definitely the conditions controlling germination, but with a view to the practical application of the same in mushroom culture.

CONDITIONS OF GROWTH.

Incidental to the other work, and as opportunity offered, a few experiments have been made regarding the conditions of growth in some of the Basidiomycetes.

Hypholoma appendiculatum and *Coprinus micaceus* have been found to lend themselves with the greatest ease to artificial cultural conditions. There is no difficulty at all in obtaining pure cultures of these forms by the ordinary methods of preparing dilution cultures. Their spores have never failed to germinate in any agar in which they have been sown. They germinate in from six to twelve hours and grow rapidly. The spores nine months old germinate quite as rapidly as

when first gathered. Little time has been devoted to the germination of the spores of *Collybia velutipes;* but from the few experiments which have been made I believe that this is another form which may be of value for cultural purposes. Heretofore, students occupied with the physiological problems of nutrition, osmotic pressures, toxicity, etc., have worked either with the algae, or with the lower fungi. There is no reason, whatever, why these studies should not be extended to include such Basidiomycetous forms as those named in this paragraph.

COPRINUS MICACEUS.

Transfers of *Coprinus micaceus* have been made from pure cultures to sterilized test tubes containing bean pods, bean stems, sugar-beet plugs, pieces of decayed wood, wheat straw in bean decoction, and wheat straw in manure decoction. Abundant growth has resulted in every case with the exception of the sugar beet. The writer has rarely succeeded in obtaining good growth with any Basidiomycete on the sugar beet as a solid substratum, although almost perfect germination occurs in a decoction of the beet.

To further test the growth of this fungus, sand was put into ordinary glass fruit jars to the depth of an inch and a half, and water was added until it stood a little above the sand. Two or three dead hickory boughs were placed in each jar, and the covers put on, the rubbers being left off to admit air. A thick piece of absorbent cotton was then tied over the top of each, and the jars sterilized. Other jars were filled to the height of 3 or 4 inches with chopped wheat straw, to which a little water was added, and a small vessel of water was placed in each jar to insure a moist atmosphere. These were covered and sterilized as described above. After some mycelium had been transferred from test tubes to these jars, the glass covers and absorbent cotton were immediately replaced. Under these conditions the mycelium grew luxuriantly; and in every instance it showed the same structure, and also the same beautiful amber or burnt orange color which have been found to be characteristic of a certain mycelium growing on the posts and other pieces of timber in mines. The sporophores of *Coprinus micaceus* have been observed in connection with this mycelium in the mines; but, if I am correctly informed, a connection between the two has never been definitely established. There can be no doubt, in the light of our cultures, that the two are united, and that this deep yellow mycelium is the characteristic mycelium of *Coprinus micaceus*.

About three weeks after these cultures were started, some sterilized sand was poured into the jars containing chopped straw, and in a few days numerous small sporophores appeared. These did not reach full development under sterile conditions, but some of them expanded at once when the absorbent cotton and the covers were removed and an

abundance of free air admitted. Some porous pots were partly filled with sand and decayed wood, others with soil and decayed wood, and sterilized. These were inoculated with the mycelium of *Coprinus micaceus* and put in a warm, moist place in the conservatory. In about three weeks sporophores appeared.

HYPHOLOMA APPENDICULATUM.

The mycelium of *Hypholoma appendiculatum* has also been found to grow vigorously in test tubes containing bean pods or stems, and on sterilized wheat straw in manure and in bean decoctions, but it has shown little or no growth on sugar-beet plugs. It developed a splendid growth of mycelium in fruit jars of sterilized straw, but no sporophores were formed.

AGARICUS CAMPESTRIS.

Considering the results obtained with the action of an artificial digestive fluid, it seemed very desirable to test the effect on the spores of *Agaricus campestris* of a passage through the animal body. Some large glass jars were partly filled with horse manure, others with chopped wheat straw, and thoroughly sterilized. After a rabbit had been kept in captivity for some days and fed only on carefully washed vegetables he was given a quantity of these spores. The gills of the fungus were wrapped in carefully washed lettuce leaves or mixed with sterilized bran and water. When the rabbit had been fed for several days on this diet alone, the animal excrement obtained on the last day was placed in the previously prepared glass vessels and sterilized sand was then added to the depth of $1\frac{1}{4}$ inches. The jars were placed under suitable conditions of light and temperature, but after having been kept for several months they yielded only negative results. The excrement of the rabbit was also put into large test tubes containing sterilized stable manure. These, too, gave no positive results. In every one of the cultures, however, species of the Coprini appeared. The most abundant form developed was probably *Coprinus solstistialis*, but it was not determined to be such with certainty.

The spores of *Agaricus campestris* which had germinated in drop cultures were frequently transferred, as already mentioned, to test tubes containing sterilized manure, wheat straw in manure decoction, wheat straw in bean decoction, bean pods, bean stems, sugar-beet plugs, manure agar and bean agar, respectively. In the test tubes containing manure there was never any further growth. This is surprising considering the natural habitat of this fungus, and can be explained only on the supposition that the manure was not obtained under proper conditions, although great care was exercised in its selection, and tests were made both with the fresh horse manure, and with manure which had been fermented by the gardener preparatory to making a mushroom bed. On the other hand, there was a consider-

able development of mycelium in the slant tubes of manure agar, and also on the wheat straw in manure decoction. This suggests that the manure may have been used in too concentrated a form.

In one test tube containing sugar-beet cylinders, there was an abundant growth of mycelium; but as a rule little development occurred on this substratum, and there was rarely any growth unless the spores had germinated in a drop of beet decoction. Of all the solid substrata used, bean stems gave invariably the best results. Photographs of two such cultures are reproduced in Pl. II. In every instance in which germinated spores were transferred to bean stems, growth resulted, irrespective of the medium in which the spores had germinated.

HISTORICAL.

According to Hoffman (1859), the germination of fungous spores was first studied by Prevost (1807) and Ehrenberg (1821). Since their publications, especially during the latter half of the past century, various investigators have occupied themselves with a study of the germination of the spores of the fungi. Inasmuch as the accounts of this work have, so far as I am aware, never been brought together, there is to-day, in the minds of students in general, a rather indefinite idea as to the present status of this study, particularly with reference to the Basidiomycetes. In view of this fact it becomes desirable to give at this time a brief summary of the results already obtained along this line.

This review will be confined exclusively to a consideration of the literature which deals with the phenomenon of germination as exhibited in the Basidiomycetes. Frequently the reports of this work do not form separate papers, and the results obtained can be determined only after diligently searching through long treatises. No attempt is made in the present paper to give an exhaustive treatment of this history; but it is doubtful if any important references have been omitted. Such empirical cultures of the Basidiomycetes as have been carried on in Italy and in other parts of the world from a very early date have recently been described somewhat in detail by Constantin (1892), and will not be considered here.

1842. Corda made the statement that the spores of the fungi germinate just as readily as do all seeds and the spores of many higher plants; but he gave no proof of this experimentally. In the same year, however, he figured in his Icones Fungorum the germinated spores of *Sphærobolus stellatus*. He did not germinate these spores by artificial means, but sketched them in the condition in which he found them where they had been scattered from the sporangia.

1853. Tulasne described and figured the germination, in water or moist air, of the spores of *Tremella mesenterica* Retx., *Exidia spiculosa* Sommerf., *Tremella violacea* Reth., and *Dacryomyces deliquescens* Dub.

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1855. Sachs studied the development of *Crucibulum vulgare*, but did not observe the germination of the spores, and said that such germination had not been obtained in artificial cultures in the Nidulariaceæ.

1859. Hoffmann published an account of his germination, in water and in moist air, of the spores of several Basidiomycetes. He discussed briefly, and illustrated the first stages in the development of the following species: *Dacryomyces deliquescens* Dub., *Lycoperdon constellatum* Fr., *L. verrucosum* Ruff. (*L. gemmatum* Batsch.), *Bovista plumbea* Pers., *Cyathus striatus*, *Tremella mesenterica* Retz., *Spathula fluvida* Fr., *Thelephora quercina* Pers., *T. uvida* Fr., *Hydnum auriscalpium* L., *Trametes suaveolens* F., *Agaricus Coprinus plicatilis* Curt., and *Agaricus velutipes* Curt. He found that the spores of Lycoperdon germinated with difficulty, their germination being the exception rather than the rule.

1860. Hoffmann continued his investigations along this line, considering to some degree the physiology of the process. He seems to have been the first to have studied to any extent the germination of Basidiomycetous spores under definite and known conditions. His experiments were so conducted that the germinating spores could easily be studied under the microscope without disturbing the cultures, but there is no evidence that any special precautions were observed to keep the cultures free from contamination. He made many temperature tests, and also studied the effects of light and various chemicals, chiefly acids, on germination. It was found that the time of year did not matter for germination, but that the spores required only the proper moisture and temperature; germination seemed to be as good in direct or diffused light as in darkness, but the spores of *Agaricus campestris* germinated earlier in the dark than in the light; spores not germinated were not killed by frost, though freezing was found to be fatal to germinated spores; spores in a state of germination were actually killed by drying, but many spores germinated better in moist air than in water; no evidence was found that weak acids favored germination, and it was concluded that a period of rest was not necessary for the germination of Basidiomycetous spores, though many of them germinated after a period of weeks and even years. He determined the length of time required for germination, and sketched the germinated spores of *Polyporus versicolor* (5 days), *P. squamosus* (6 days), *Coprinus micaceus* (1 day), *Psathyra stypticus* (3 days), *Mycena vulgaris* (2 days), *Collybia Oreades* (3 days), *C. conigenses* (3 days), *Coprinarius papilionaceus*, *Thelephora hirsuta*, and *Exidia Tremella granulosa*. In this later work he was unable to germinate the spores of Lycoperdon.

1861. La Bourdette, in a note regarding his methods of culture, claimed that after several years of experimentation he had succeeded in developing the edible Agaricus from spores by means of potassium nitrate and without the use of manure. The nitrate was put into the soil with the spores of the agaric to the depth of from 3 to 4 mm. The soil was composed largely of calcium sulphate beaten down and nothing added. Under these conditions an indefinite growth of the fungus was obtained. According to his statements, the mushrooms derived by the method just described attained an average weight of 600 grams, while those obtained by the ordinary complicated methods of culture averaged but 100 grams.

1861. Chevreul presented to the Academy of Science, Paris, some mushrooms that had been grown by La Bourdette. La Bourdette had sown the spores in sand and water on a glass plate. The most vigorous plants thus obtained were later transferred to a humid soil composed of vegetable earth. This soil was placed in a cave and covered with sand and river gravel to a depth of .25 m., this being again covered with broken plaster .15 m. in depth. The bed was watered with water containing 2 grams of potassium nitrate to a square meter

of the soil. Under these conditions the fine group of sporophores sent to the academy had developed in six days.

These results seem to have been accepted with a grain of salt, as it were, even by some of La Bourdette's contemporaries.

1863. Nylander found something of the fabulous in La Bourdette's reports, and believed that his fungi had come up by chance, having no connection whatever with the spores that had been sown. He had never himself been able to germinate the spores of *Agaricus campestris* in cultures, and had found no evidence in literature of such germination. He cultured these spores many months in hot, moist chambers, and on different substrata, but chiefly on horse manure, and never saw even the beginnings of germination.

The gills of *Agaricus campestris*, and the water in which the fungi had been washed when prepared for food, were scattered in places where this fungus often chanced to come up, but they never produced the plant. This was repeated for many years, but always with negative results. He thought it a matter of great interest to determine the method of germination of these spores and the physiology of the process, and he suggested that by an easy experiment, but one of great weight, it might be shown whether the spores of *Agaricus campestris* require, before they will germinate, to be subjected to such a digestive process as that which takes place in the animal intestines.

1865. De Bary referred to the germination of *Coprinus fimetarius* which produced mycelium and fruit in a proper nutrient medium, and also spoke of the germination of the spores of the Tremellineæ and of Dacryomyces.

1866. De Bary reported much work done on the germination of the Phycomycetes and Ascomycetes, and figured such germination; but he seems to have attempted at this time the germination of only one Basidiomycete, *Phallus impudicus*, and to have failed in this.

1867. Woronin, according to De Bary, germinated the spores of *Exobasidium vaccinii*, and determined the length of a generation to be fourteen days under the most favorable conditions; that is, on the leaves of Vaccinium.

1870. Pitra, twenty-eight years after Corda had recorded his observations on germination in *Sphaerobolus stellatus*, found the germinated spores of this species in the sporangium and figured them. He observed nothing peculiar in this germination, and noted that the hyphæ might grow from one or both ends of the spore.

1872. Woronin determined that the spores of *Coprinus ephemerus* would germinate in six hours in a thoroughly cooked and filtered decoction of horse manure.

1874. Hartig, as reported by De Bary, described in full the development of *Agaricus melleus*.

1875. Eidam demonstrated the easy germination, in drops of manure decoction, of the spores of all the Coprini which he had studied. Spores of *Agaricus coprophilus* germinated in from eighteen to twenty hours and conducted themselves exactly like those of the Coprini. He made the interesting observation that in spite of like conditions not all of these spores germinated at the same time. In some cases germination did not take place until after four days, and then short, thick tubes were put out.

1875 and **1876.** Van Tieghem who, like Eidam, was seeking to demonstrate the presence of sexual organs in the Basidiomycetes succeeded in germinating the spores of several Coprini, *Agaricus coprophilus*, *Hypholoma fasciculare*, *Pholiota mutabilis*, *Galera tener*, *Collybia velutipes*, and many forms with white spores. The stages of development could be easily traced in all, but showed most beautifully in *Collybia velutipes*.

1876. Hesse published a paper on the germination of the spores of *Cyathus striatus*. He did not accept Hoffman's germination of the spores of this species, and said that now, sixteen years after the appearance of Hoffman's report, there

was recorded for the first time the real germination of this form. Spores were collected at various times of the year, and cultures made in water, in nutrient solution, and on sterilized rabbit manure, but no germination occurred. The same methods were tried with *Geaster*, *Borista*, *Lycoperdon*, *Tulostoma*, *Scleroderma*, etc., but with these, also, they resulted in failure. It was thought that the spores of the Gasteromycetes might first be capable of germination after passing through the animal body, and experiments to determine this point were tried, but gave only negative results.

At last he succeeded in bringing the spores of *Cyathus striatus* to germination; but he did not wish to describe his methods until he had convinced himself that he had found not only the way to germinate these spores, but the spores of other Gasteromycetes as well. This much, however, he ventured to reveal, that these spores would germinate in pure water in from eighteen to twenty-four hours if the other necessary conditions for germination had been fulfilled.

1876. Eidam's publication, in which the germination of the spores of *Cyathus striatus* and *Crucibulum vulgare* was described and figured, appeared almost simultaneously with Hesse's paper. Eidam considered that the negative results obtained by the earlier students of these species were due to the fact that the spores had not been brought into conditions favorable for their germination and further development prior to the setting up of the cultures. In order to eliminate this source of error the spores were placed in suitable nutriment under a bell jar lined with moist blotting paper and kept at a temperature from 20–25° C. for a day and a night. Thus the life activity of the spores was enhanced. Those kept at the room temperature from October to December gave results much more slowly and quite irregularly.

Decoctions of plums or prunes, bark, decaying wood, hay, and horse manure, filtered and crystal clear, were used as culture media. Due precautions were exercised to obtain the spores in as pure a condition as possible. The spores of *Cyathus striatus* germinated in a decoction of manure at a temperature of about 25° C. One, two, or three germ-tubes were formed. The spores were also germinated and abundant mycelium developed on sterilized horse manure. Several germ tubes were developed from each spore in *Crucibulum vulgare*, and the spores swelled to twice their normal size before emitting tubes.

1876. Reese added his name to the list of those who had germinated the spores of the Coprini. He affirmed that he had tried in vain to get positive results with *Agaricus campestris*, *Sphærobolus stellatus*, and *Crucibulum vulgare*, and that he had at last turned to *Coprinus stercorarius*. This species germinated in from four to five hours in a sterilized decoction of horse manure; and, in the presence of a high temperature and much moisture, fruit bodies were formed in eight days. If kept cooler and drier the length of a generation was from eighteen to twenty days. Fruit bodies of *Coprinus ephemerus* were never obtained in less than twenty-four days after the germination of the spores.

1877. Brefeld's first important work on the Basidiomycetes appeared, as is well known, in 1877. He succeeded at this time in obtaining the early stages of development for a considerable number of species.

Spores of *Coprinus stercorarius*, *C. ephemerus*, *C. ephemeroides*, and *C. lagopus* germinated easily and surely in a decoction of manure. Sporophores of *Agaricus mellcus* were placed in a watch glass for a quarter of an hour. Some of the spores thus obtained were transferred to a drop of fruit decoction, where they germinated in three days. According to his investigations, the spores of the Nidulariaceæ germinated readily at a temperature of 15 to 18°. *Typhula variabilis* and *T. complanata* were studied, and their spores germinated as representatives of the Clavariaceæ. The spores of *Tremella* were easily

and surely germinated, but only when freshly thrown off. In the case of *Dacryomyces deliquescens*, the spores germinated, after several divisions, with conidia formation. Conidia were formed in all the species studied, but only those of Tremella were observed to germinate. Many Geasters and Lycoperdons were cultured, but the spores of none of them were brought to germination.

1881. Brefeld has given a detailed account of his culture methods in the fourth part of his studies of the Fungi. It would not be practicable to enter upon a discussion of those methods here. They are familiar to all students of mycology at the present day. We might, however, remark, in passing, that he used a great variety of decoctions, and evidently employed sterilization precautions wherever possible throughout his work.

1884. De Bary gave a partial summary in 1884 of the results obtained previous to that time in the germination of Basidiomycetous spores, but he contributed little to the subject that was really new. He considered the external conditions necessary for the germination of fungous spores to be a supply of water, a supply of oxygen, a certain temperature, and in some cases a nutrient substance or food supply. He made the statement later, however, that the necessity of oxygen for the germination of these spores had never been sufficiently tested. Of the fungi whose basidiospores had never been certainly known to germinate he cited *Agaricus campestris*, the Lycoperdaceæ, Phalloideæ, and Hymenogastreæ.

1884. Fischer studied the life history of *Sphærobolus stellatus*. Very beautiful cultures were obtained by sowing sporangia on sawdust that had been boiled in water and afterwards put into porous clay dishes. After the sporangia were sown the pots were set into water that the cultures might be kept moist. As many as six fruit-bodies, measuring from 9 to 9½ cm., were developed in a single pot at one time. Spores kept from the end of February to the following October germinated, but more slowly than fresh spores.

Gemmæ and basidiospores were found inside the same sporangium, and the germination of both was observed and figured. For a long time he was not able to induce the spores to germinate. High temperatures, feeding to birds, etc., were tried, but always with negative results. He had almost come to the conclusion that these spores were not capable of further development when they were found to germinate in less than twenty-four hours in a culture of manure decoction free of gemmæ. Each spore was observed to emit a single germ-tube, whereas Pitra (1870) found tubes at both poles of the spores. For this reason Fischer thought that Pitra did not see the basidiospores germinate, but the gemmæ instead, as these send out hyphæ from both ends. He also doubted that Corda (1842) saw these spores germinate. Many of the spores do not germinate, but gradually disintegrate; and he suggests that in this species the gemmæ have assumed the function of the spores, and produce mycelium at the cost of the true basidiospores which are used to nourish the growing hyphæ.

1888 and **1889.** Brefeld published parts seven and eight of his studies of the Fungi in 1888 and 1889, respectively. These volumes are devoted almost exclusively to a consideration of the development of the Basidiomycetes. In them the author describes the germination of more than one hundred and sixty species, and mentions over forty others which were cultured but failed to germinate. The very interesting discovery was made that while the spores of *Panæolus campanulata* germinated only in manure decoction, the mycelium grew abundantly in all the nutrient solutions in which the spores would not germinate. This was also said to be true for the Coprini, and for almost all the manure-inhabiting Agarics.

1891. Constantin germinated the spores of *Nyctalis lycoperdoides* in pure cultures on all sorts of nutrient substances—oak and beech leaves, potato in orange juice, beet, carrot, and on other mushrooms. The spores germinated in twenty-four hours at a temperature of 24°, and later gave rise to normal fruit-bodies. He was able to keep cultures of this species for three years without contamination.

Marasmius olex was also cultured. Olive leaves covered with small plants were put on a plate and placed under a bell-jar. When the fruit-bodies appeared, he placed sterilized watch glasses, containing sterilized water under the caps. Basidiospores were thus gathered and transferred by means of a platinum needle to test-tubes containing sterilized olive leaves. After some time, sporopores of the *Marasmius* appeared in these tubes.

1892. Scholz was able to trace every step in the development of *Agaricus melleus*, and, according to his reviewer, he observed much that was new.

1894. Hartig demonstrated the fact that the spores of *Merulius lachrymans* germinate, in cultures, only when ammonia or the salts of sodium or potassium are added to the infusion in which the spores are placed. He did not believe that these salts were to be regarded as nutritive in their effect, but that they merely served to render possible the removal of the spore pellicle that covers the germ aperature. He was of the opinion that every spore and every seed contains a certain quantity of nourishment derived from the parent plant, and instantly available for use in germination; hence an external supply of food can not be necessary.

1894. Constantin's paper on the culture of *Polyporus squamosus* can be mentioned only by title, as the writer has been able to obtain neither the original article nor any review of it.

1895. Wehmer brought some frozen specimens of *Pleurotus ostreatus* into the laboratory after about two weeks of continued frost. The plants were gradually thawed out, and the spores collected. Somewhat later these spores were found to germinate abundantly in sugar solution.

1898. Repin published a bulletin which contains this statement (quoted from a reprint in English): "There is no mystery in the germination of mushroom spores. It can be brought about on any of the nutrient media used by bacteriologists, but germination does not take place with rapidity, and requires to be stimulated by some artificial means or contrivances, which vary with the ideas of the operator, and which may be discovered after some fruitless experiments have been made" This lucid statement can not be said to contribute greatly to our knowledge of the subject in hand.

Judging from Repin's sentence, "The spores that will germinate, and these are always the fewest in number, do so by—," it would appear that the students of the Pasteur Institute had not succeeded in obtaining a high degree of germination with the spores of *Agaricus campestris*.

1901. Duggar, in his recent investigations of the germination of fungous spores, studied but four Basidiomycetes—*Coprinus micaceus*, *C. comatus*, *C. fimetarius*, and one species of *Boletus*. Of these, *Coprinus comatus* and *Boletus* showed no germination; *Coprinus fimetarius* gave small percentages of germination in plant decoctions only; and *Coprinus micaceus* gave almost perfect germination in bean and dung decoctions, but scarcely any germination in solutions devoid of plant decoction. The mycelium of *Coprinus micaceus* was found to grow abundantly in solutions in which the spores failed to germinate. The conclusion was therefore drawn that, if the stimulus to germination be a food stimulus, it must belong to the class of peculiar foods.

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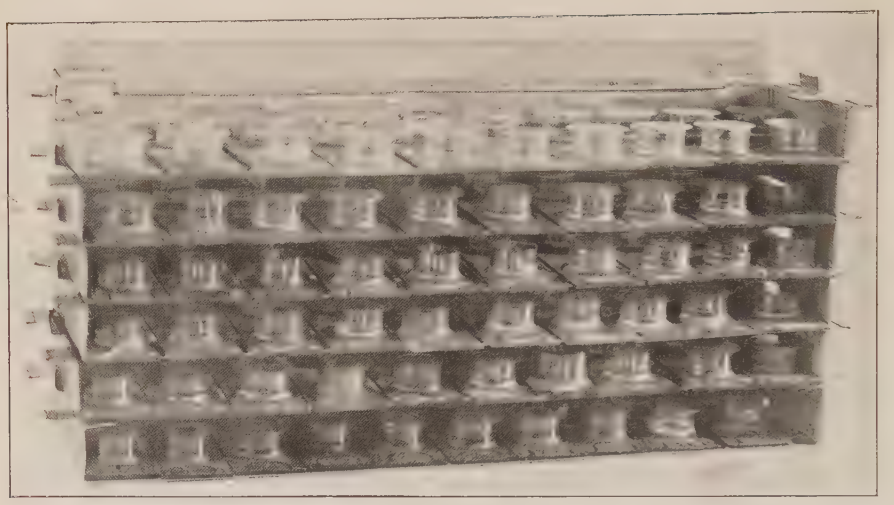
AGARICUS CAMPESTRIS, GERMINATED SPORES.

MYCELIUM OF AGARICUS CAMPESTRIS.

Some spores were germinated in a hanging drop and then transferred to bean stems in test tubes. The photograph was taken by Dr. B. M. Duggar ten days after the spores had been transferred to the tubes.



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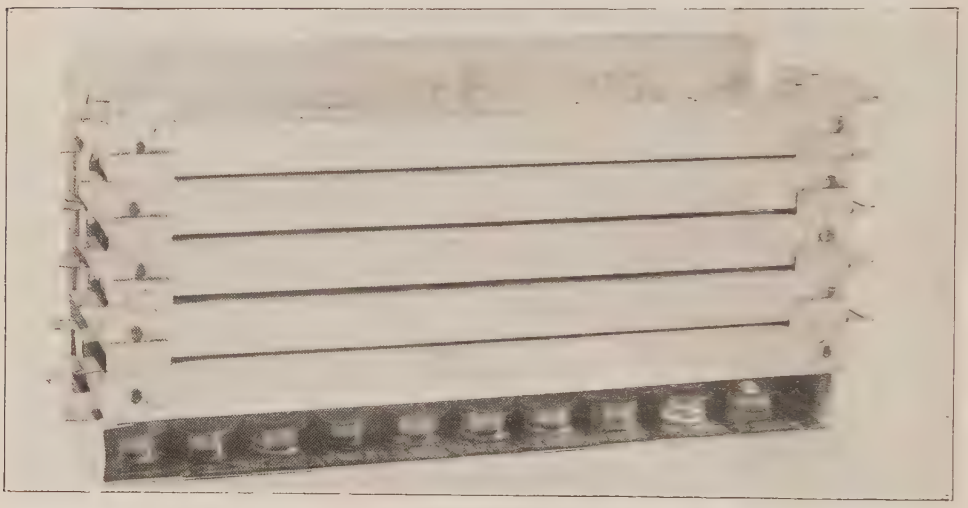
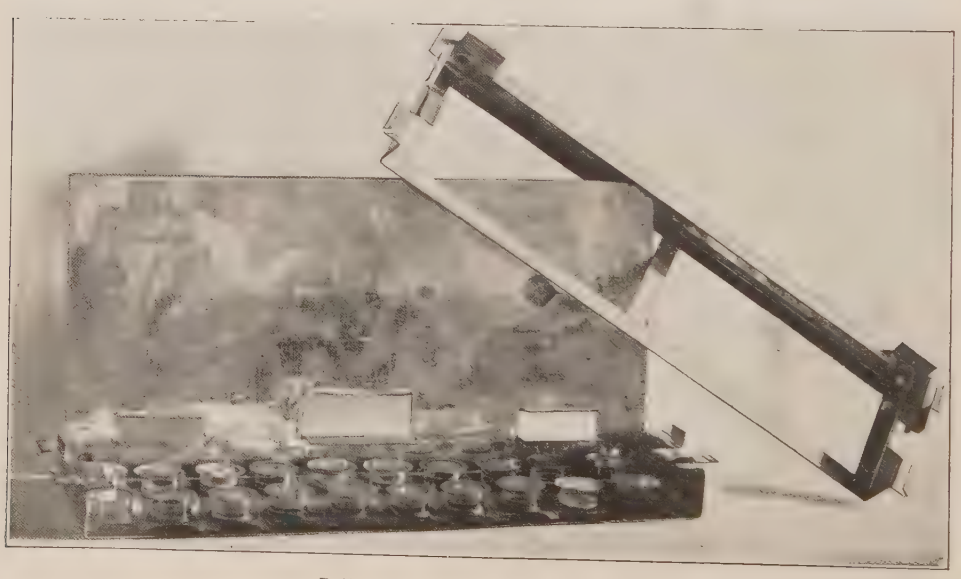


Stand closed.



Bottom tray and one of the other trays.

STAND FOR SUPPORTING VAN TIEGHEM CELLS.

EXPLANATION OF PLATES.

PLATE I.—AGARICUS CAMPESTRIS.

Fig 1. The germinated spores as they appear when but a few spores have germinated in a given culture. In bean decoction.

Fig. 2. The germinated spores as they appear when large numbers of spores germinate under the influence of the growing mycelium of Agaricus campestris. These spores had been kept for a week after their germination, but showed no evidence whatever of further growth. In Lepiota decoction.

Fig. 3. One of the spores shown in fig. 2, twenty-four hours after it had been transferred to a drop of fresh Lepiota decoction.

Fig. 4. Some spores which had germinated in manure decoction, showing exactly the same appearance as those in fig. 2. They were kept for one week after germination, but showed no signs of further development, and were then transferred to a drop of fresh manure decoction. They were figured twenty-four hours after the transfers were made. It will be noted that growth was much slower than in the Lepiota decoction. (Fig. 3.)

Fig. 5. Germination and growth in manure decoction containing hippuric acid.

Fig. 6. A germinating spore showing the irregular, much-branched hyphæ characteristic of the first stages of growth. In beet decoction.

Fig. 7. The outer ends of two threads showing the characteristic structure of the older mycelium. In Lepiota decoction.

PLATE II.—MYCELIUM OF AGARICUS CAMPESTRIS.

Spores germinated in a hanging drop, and then transferred to bean stems in test-tubes.

PLATE III.—A STAND FOR SUPPORTING THE VAN TIEGHEM CELLS.

Fig. 1. The stand open, as when one is examining the cultures.

Fig. 2. The stand closed and ready for placing in the thermostat.

Fig. 3. The bottom tray and one of the other trays, showing structure.

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APPENDIX.

A CONVENIENT STAND FOR HOLDING SLIDES IN CELL-CULTURE WORK.

A stand for supporting the slides, when one is using the Van Tieghem cells, should be made of such material and in such a way that it will neither burn, warp, nor melt upon being heated, for it is often desirable to sterilize the cells before making up the cultures. It should also combine economy of space with ease of manipulation. All these points are characteristic of the little piece of apparatus which I have used, three views of which are shown in Plate III.

This stand consists, as will be seen from the illustrations, of a series of trays placed one above another. Each tray was made from a single piece of tin without the use of solder. The tin measured 13¼ by 3⅞ inches after the edges were turned. This was folded on three sides, just as one folds a piece of paper in making the boxes described by Lee (1896) for embedding material in paraffin. A strip 1⅛ inches wide along both ends and on one side was bent up at right angles to the rest, so that a box, open at the top and along one side, was formed, which measured 11 by 2¾ inches on the bottom. The double, triangular, ear-like projections formed at the two corners were folded along the back and secured by means of rivets. The tin was then cut or slashed three-eighths of an inch deep 1 inch distant from either corner on the back. Similar cuts were also made at the corners, and three, equally distant from each other and from the outer edges, were made on either end. The segment of tin along the middle of the back and those next to the free, outer segments on the ends were folded in until parallel with the bottom. These act as a shelf on which to rest the next higher tray. The outer and innermost segments at both ends were bent outward, forming projections which are very useful in lifting the trays. The second segment from the corner on either end was bent out at right angles to the side, and then the outer portion of it was again turned up until it was parallel with the position which it formerly occupied. These, with the segments at both corners along the back, which were left erect, prevent the next higher tray from slipping or sliding. It was found desirable to cut the bottoms of the trays out, as shown in fig. 3, since the rapid absorption of heat by the tin has a tendency to increase the condensation moisture on the cover glasses.

For convenience in use, it is necessary that the trays be about one-fourth of an inch narrower than the slides are long. Unfortunately the slides thus extending over the edges of the pans are very easily struck, and the cultures thereby endangered when one is putting other material into or taking it out from the thermostat. To guard against such accident, as well as for greater ease in carrying, a bottom tray was made one-fourth of an inch wider than the others, and with a back 5½ inches high. This tray had five segments cut at each end, instead of four, and these were turned the same as in the other trays except that the outermost was bent in to give greater stability. Shelves were made along the back by cutting and folding in the tin at three points (fig. 3). The windows thus formed give free circulation of air. These windows, each 2½ inches long and 1 inch deep, were so cut that if the pieces of tin freed

along three sides had been bent straight inward, they would have formed shelves one-fourth of an inch higher than those at the ends. But they were doubled in close against the back for one-fourth of an inch, and then turned out until they stood parallel with the bottom of the tray. This gives a little back at the points where the windows occur and prevents any cultures on the second tray from slipping through these open spaces. For convenience in handling, the bottom was not cut from this first tray as from the others, and it may be used as a support for cultures or not, at the discretion of the investigator.

The trays were all made of the same size. Five trays besides the bottom one constitute "a set" as we have used them. Each set holds 120 cultures and occupies only 36 square inches of space in the thermostat. The trays may, of course, be made of any length or of any height, the dimensions given being those best suited to the thermostat which we have used. When all the trays have been filled in making up a set of cultures (fig. 1), the five upper ones are lifted together and so placed on the lowest pan that their open sides are against the back of this tray (fig. 2).

The number of words which seem to be necessary for accurately describing this little piece of apparatus makes it appear somewhat complicated; but if one will take a piece of paper of suitable dimensions and follow the description given, he will find that the making of a model for one of these trays is a very simple matter.

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